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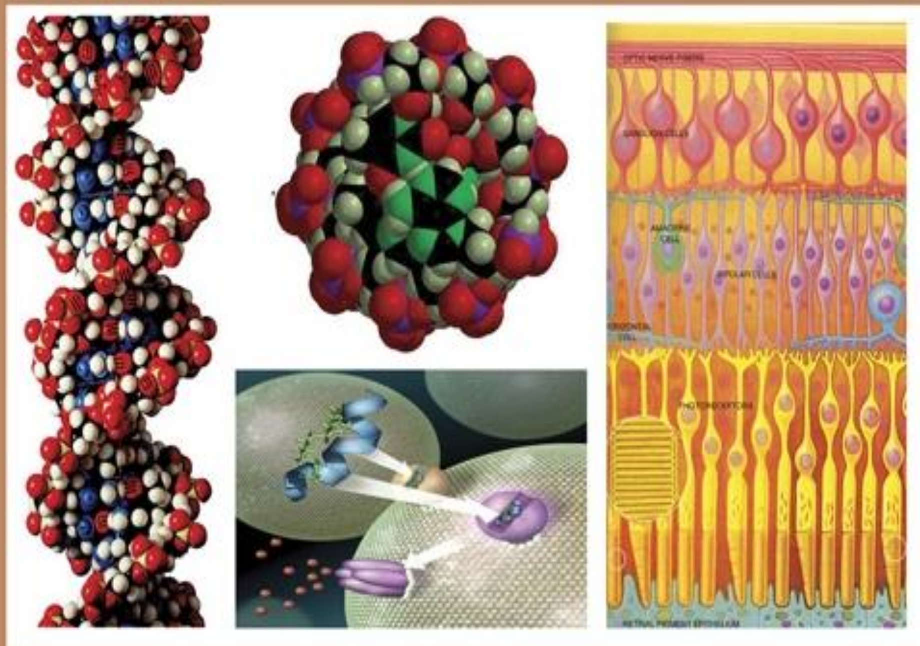

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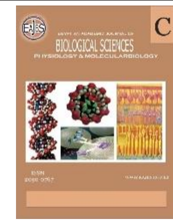

A potential Role of Metformin in Hepatocellular Carcinoma: An In Vitro Study of LncRNA-AF085935/Glypican-3/MAPK Pathway

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ABSTRACT

Background: Hepatocellular carcinoma is on the rise globally. The anticancer effects of metformin have been mainly studied in the context of supra-pharmacological concentrations (>1 mM). In this study, we aim to investigate the effects of therapeutic doses of metformin on the lncRNA-AF085935/glypican-3/mitogen-activated protein kinase pathway in HCC. **Methods:** HepG2 cells were treated with various concentrations of metformin (0, 5, 10 and 20µM) for different durations (24, 48, and 72h). Cell viability was evaluated by MTT assay, while gene expression levels of lncRNA-AF085935, glypican-3, VEGF, P53, NFκB genes were evaluated by quantitative real-time PCR. The ratio between the phosphorylated p44/42 and the total p44/42 protein level was assessed by western blot. **Results:** Compared to the control groups, we found a significant decrease in cell proliferation, lncRNA-AF085935 gene expression (p value <0.001), VEGF gene expression (p value <0.001) and p-p44MAPK protein level (p value <0.0001) in all metformin-treated groups except the metformin 5µM-24h group. Furthermore, our data showed a significant decrease in gene expression for both glypican-3 and NFκB at 48 and 72h compared to the control group (p value <0.001, 0.001) respectively, with no significant effect of metformin after 24h. Also, we found a significant increase in p53 gene expression in 20µM group compared to the corresponding control cells of the same duration, while the 5µM group showed no significant difference in any duration. **Conclusion:** Our research gives new insights into metformin's anti-carcinogenic effect on HCC through its impact on LncRNA-AF085935/GPC3/MAPK pathway.

INTRODUCTION

Hepatocellular carcinoma (HCC) characterizes more than 75% of the primary liver cancers, is the sixth most predominant cancer, and is the third most frequent cause of cancer-related mortalities across the world, according to GLOBOCAN, 2020 (Chakraborty and Sarkar, 2022). HCC mortality is anticipated to double by 2040 worldwide (Foreman *et al.,* 2018). Thus, there is high interest in finding new diagnostic and curative approaches to HCC. Risk factors that are attributed to HCC are chronic infections with hepatitis B and C viruses, factors that have been declining thanks to mass vaccination programs (Rich *et al.,* 2020).

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Diabetes, non-alcoholic fatty liver disease, alcohol, aflatoxin-contaminated food, smoking, and immune diseases are all rising risk factors (London *et al.,* 2018). Metformin is a frontline therapy for new cases of type 2 diabetes mellitus. In addition, this inexpensive drug has been known as a strong anticancer agent with variable effectiveness among different cancers or populations (Feng *et al.,* 2022). Metformin has been shown to lower the risk of developing HCC in diabetic patients (Cunha *et al.,* 2019) and to improve survival in HCC cases (Schulte *et al.,* 2019). A substantial body of evidence suggests the idea that the anti-tumoral effect of metformin is related to its ability to deactivate RAS/RAF/MAPK and PI3K/AKT/mTOR signaling pathways and down-regulate other tumor-promoting molecules (Cejuela *et al.,* 2022).

Mitogen-activated protein kinase (MAPK) pathways include three main subfamilies (Yu *et al.,* 2020); the extracellular signal-regulated kinases (ERKs), which include a rising member; ERK1/2 pathway, synonymously known as phospho-p44/42 MAPK pathway (Lei *et al.,* 2017) ,which is usually activated by growth factors and mitogens, resulting in activating cell proliferation and endurance (Lee *et al.,* 2020). The other subfamilies include p38 and the Jun N-terminal kinases (JNK) MAPKs. They are generally activated by cellular stress and inflammatory cytokines (Yu *et al.,* 2020), and their role in promoting cancer is less univocal (Rovida and Tusa, 2022).

Glypican-3 (GPC3) is a cell surface proteoglycan that is abundant in the fetal liver but not in the adult liver (Zheng et al., 2022). Interestingly, GPC3 is frequently upregulated in HCC, promoting its growth and invasiveness, but is absent in non-malignant hepatic lesions (Wu *et al.,* 2015).

In the past decade, dysregulated long noncoding RNAs (lncRNAs) have participated extensively in the pathogenesis of many cancers. LncRNA–AF085935 is an oncogene that promotes the growth of HCC cells through activating GPC3, so it is coexpressed in HCC cells and tissues (Sabry *et al.,* 2019) .To be hinted at, transcriptionally oriented, lncRNA–AF085935 is antisense to GPC3 (Lu *et al.,* 2015). Thus, the LncRNA-AF085935/GPC3 axis has gained quite some interest as a target to be studied in HCC (AL-Noshokaty *et al.,* 2022), (Sherif *et al.,* 2020). It is worthy of note that the activation of the mitogenic pathway p44/42 MAPK depends on IGF-II and GPC3 (Cheng *et al.,* 2008).

As metabolic pathways can be new therapeutic anticancer targets, we investigated the impact of low doses of metformin on the LncRNA-AF085935/GPC3/MAPK signaling pathway in HepG2 cells.

MATERIALS AND METHODS

1. Cell Culture:

HepG2 (Minnesota, USA), a human hepatocellular carcinoma cell line; was provided by VACSERA (voucher number; 00/32287) in T-75 flasks, then routinely cultured in RPMI containing glutamine 2mM (Bio west, Nampa), 10% fetal bovine serum (FBS) (PAA, Austria), and antibiotics (penicillin and streptomycin 1%) (Lonza, Belgium). The cells were incubated for 24h as a pre-culture in 5% CO2 at 37^0C. Then the cells were trypsinized and then centrifuged. Cell pellets were subdivided as shown in Figure 1. Metformin (Sigma-Aldrich Co., U.S.A., CAS n1115-70-4) was freshly dissolved in 2% dimethyl sulfoxide (DMSO) as a vehicle and prepared in different concentrations (5, 10, and 20μM).

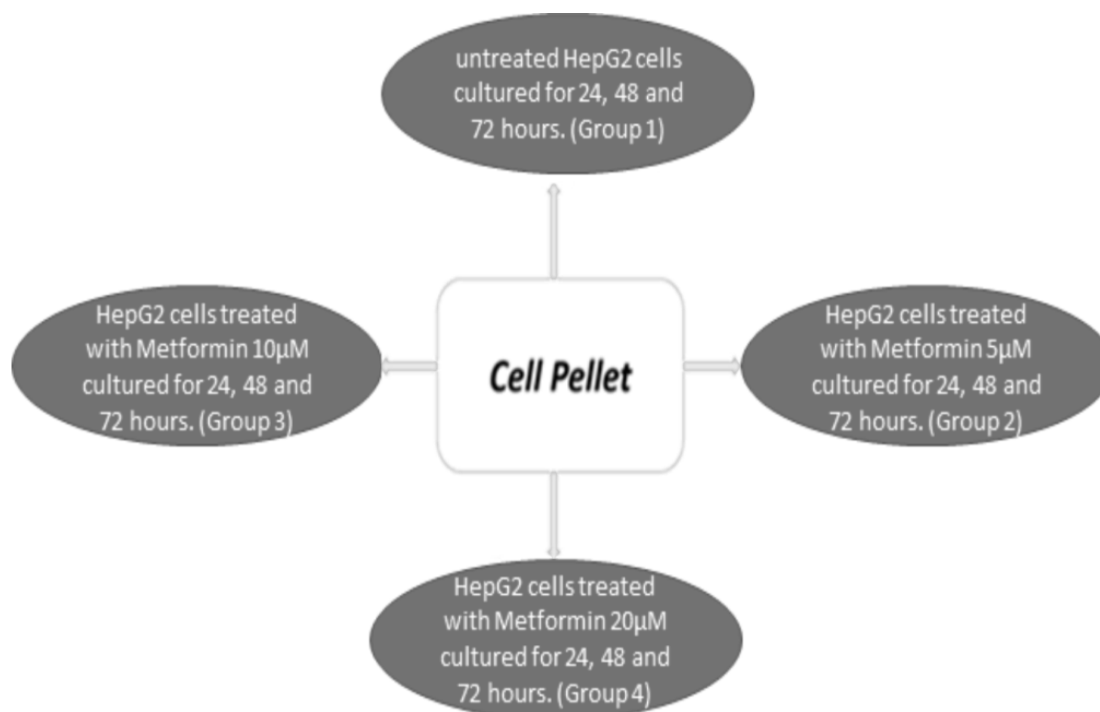

Fig. 1: Cell culture groups**.**

2. Cell Viability Assay (MTT assay):

We fixed HepG2 cells into 96-well plates for 24h. Then, culture media were changed with new media supplemented with different concentrations of metformin for 24h, 48h, and 72h. For each well, the culture medium was replaced by 100µL of serum-free media and 10µL of MTT reagent and left for 4h, and then 100µL formazan (Biospes, China) replaced the medium. After incubation, we measured the absorbance in each well at 570 nm with the use of an ELISA reader (Stat Fax 2200, Awareness Technologies, USA).

3. RNA Extraction and Real-Time PCR:

RNA extraction and purification were done (Thermo Fisher Scientific Inc., Germany). At 260 and 280 nm, the total RNA yield was established using a Beckman dual spectrophotometer. Real-time PCR (StepOne, Applied Biosystem, USA) was used to assess gene expression using Vivantis, ViPrimePLUS One Step Taq RT-qPCR Green Master Mix I with ROX (SYBR Green Dye). Each target gene was normalized relative to the mean cycle threshold (CT) values of the housekeeping gene using the $\Delta\Delta Ct$ method. Table 1 shows primer sequences for all the studied genes.

Table 1: Primers sequence of the studied genes:

Gene	Sequence of Primer from 5′- 3′ F: Forward primer, R: Reverse primer
Glypican-3	F: GTCCCTGAACGCGACTATTT R: AGCTTGTGCCAGCTCTTT
lncRNA-AF085935	F: CAGGGCAGCAAGGTGTTTTC R: TTGGTGGGTTGCCTGATACC
VEGF	F: CGGGAACCAGATCTCTCACC R: AAAATGGCGAATCCAATTCC
NFκB	F: CCGTGTAAACCAAAGCCCTA R: CAGAGGGACAACAGCAATGA
P53	F: GCAACGGAACACTCTCCATATTTT R: CAGAGAAGAAACGGTAGCAGAAGA
GAPDH	F: GGTGGTCTCCTCTGACTTCAACA R: GTTGCTGTAGCCAAATTCGTTGT

4. Western Blot:

For all samples, the following were performed; total protein extraction (ReadyPrepTM, Bio-Rad Inc.), quantitative protein analysis (Bradford Protein Assay, Bio-Basic Inc., Canada), loading with 2x Laemmli sample buffer, electrophoresis by SDS-PAGE (FastCast™, Bio-Rad Inc.), and blotting (Bio-Rad Trans-Blot Turbo). Then the membrane was blocked at room temperature. The primary antibody of p-p44/42 MAPK (Cell Signaling Technology, #9102) was diluted and incubated overnight at 4 °C against the blotted protein. The secondary antibody (Goat anti-rabbit IgG-HRP-1mg Goat mab, Novus Biologicals) was incubated against the blotted protein for 1h at RT. A CCD camera-based imager was used to record the chemiluminescent signals (ClarityTM, Bio-Rad). Image analysis software used beta-actin (housekeeping protein) in the control sample to determine the band intensity of the target proteins.

5. Statistical Analysis:

The statistical model 18 of the SPSS software (SPSS Inc, USA) was used to plan, tabulate, and analyze the data statistically. The mean and the mean ± standard deviation (SD) were considered for quantitative data. As a measure of normality, the Kolmogorov–Smirnov test (KS) was conducted. The Mann–Whitney U test was used as a test of significance as the variables were not distributed normally. Significance was considered at $p \leq 0.05$ to clarify the results of the tests of significance.

RESULTS

Microscopic pictures of cultured cells showed decreased cell viability and enhanced apoptosis of HCC upon different concentrations and durations (Fig. 2). MTT assay was utilized to assess the viability of HCC cells in vitro. After 24h, HepG2 cells supplemented with metformin showed a significant decrease in cell multiplication in all metformin-treated groups compared to the control group (p value <0.001) except the metformin 5µM-24h group (p value >0.05).

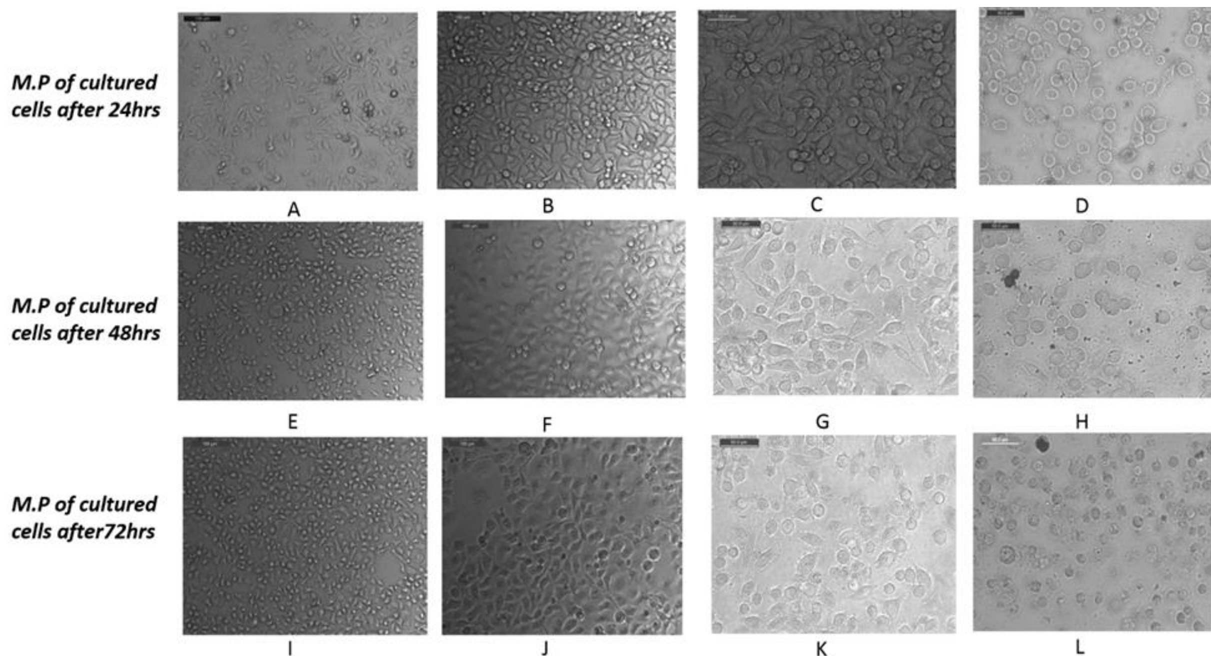

Fig. 2: Microscopic pictures of different HepG2 cancer cells.

(A, E and I): control cells cultured for 24, 48 and 72h respectively: Cells were viable, confluent (90-100%) and spindle in shape.

(B) Cells treated with 5µm Metformin cultured for 24h: cells were viable, confluent (90-100%) and spindle in shape. **(C) Cells**

treated with 10µm Metformin cultured for 24h: cells were viable and confluent (80%). **(D) Cells treated with 20µm Metformin cultured for 24h:** cells were viable but less confluent (50%) and cells were rounded in shape (indicating apoptosis).**(F) Cells fed with 5µm Metformin cultured for 48 h:** cells were viable and started to be rounded in shape. **(G) Cells fed with 10µm Metformin cultured for 48 h:** cells were viable, less confluent (50%) and they were almost rounded in shape. **(H) Cells fed with 20µm Metformin cultured for 48 h:** All cells were rounded in shape (indicating apoptosis).
(J) Cells treated with 5µm Metformin cultured for 72h: shows viable, less confluent cells (80%) and rounded in shape. **(K) Cells treated with 10µm Metformin cultured for 72h:** shows cells almost rounded in shape. **(L) Cells treated with 20µm Metformin cultured for 72h:** shows unviable cells, almost rounded in shape (indicating apoptosis).

Adding to that, we noticed that the 10 and 20µM treated cells after 24 and 48h but not after 72h were significantly lower than the corresponding 5µM of the same duration (p value <0.001) (Fig. 3)**,** suggesting that increasing the dose of metformin treatment can suppress cell proliferation rather than increasing its duration.

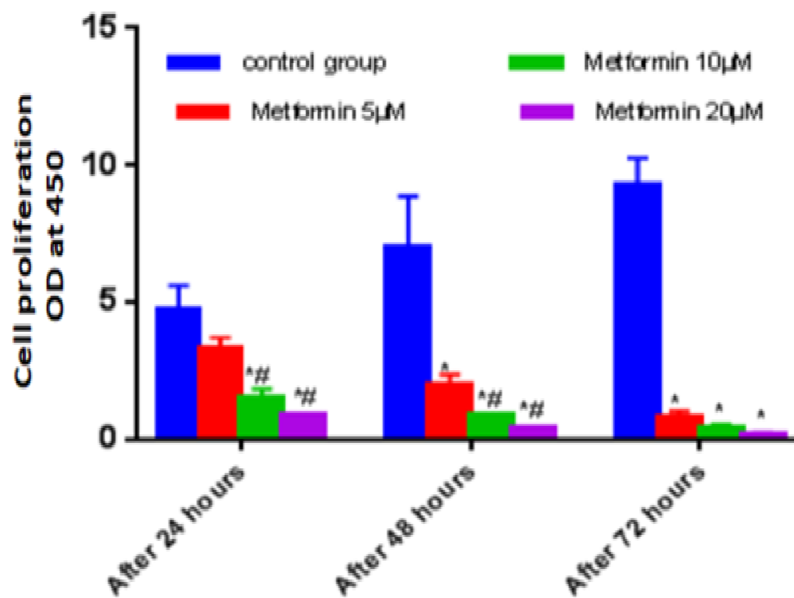

Fig. 3: Cell proliferation, mean ± SD levels among various studied groups. *(*) Denotes a significant difference (p value <0.05) against control group of the same duration. (#) Denotes a significant difference (p value <0.05) against Metformin 5µMgroup of the same duration.*

GPC3 showed a significant decrease in all the studied groups (compared to the control groups) for 48 and 72h (p value <0.001) but not for 24h treated cells (Fig. 4)**,** highlighting that increasing the duration of metformin treatment can suppress the GPC3 expression. LncRNA-AF085935 expression levels exhibited a significant decrease in all treated groups (p value <0.001) except for 5µM-24h group (p value >0.05).

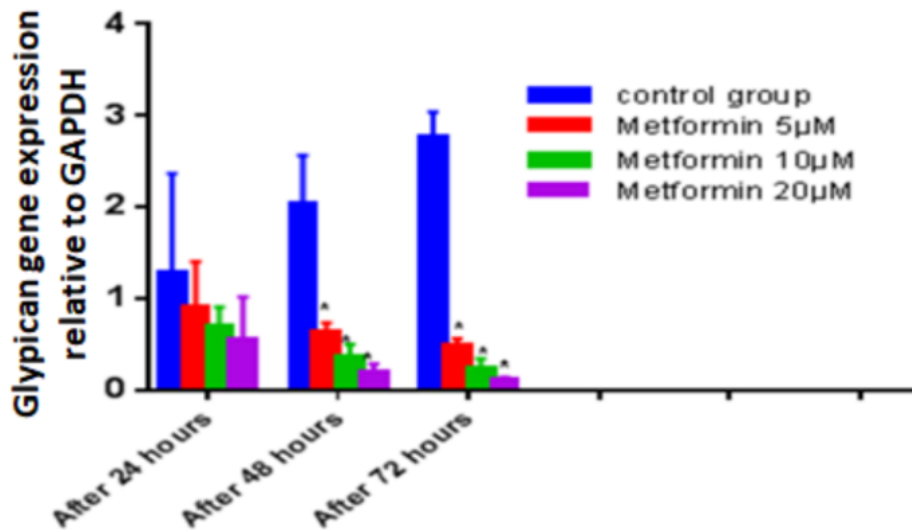

Fig. 4: Glypican-3 relative gene expression. *(Mean ± SD) among various studied groups. (*) Denotes a significant difference (p value <0.05) against control group of the same duration.*

In addition, its expression levels in each 20µM treated cell were significantly reduced compared to the lower concentrations of the same duration (Fig. 5**),** suggesting that metformin can suppress LncRNA-AF085935 expression in a dose- and duration-dependent approach.

For further study of the LncRNA-AF085935/GPC3 axis, we performed a correlation between the LncRNA-AF085935 and GPC3 and found a strong positive correlation ($p < 0.001$) (Fig. 6).

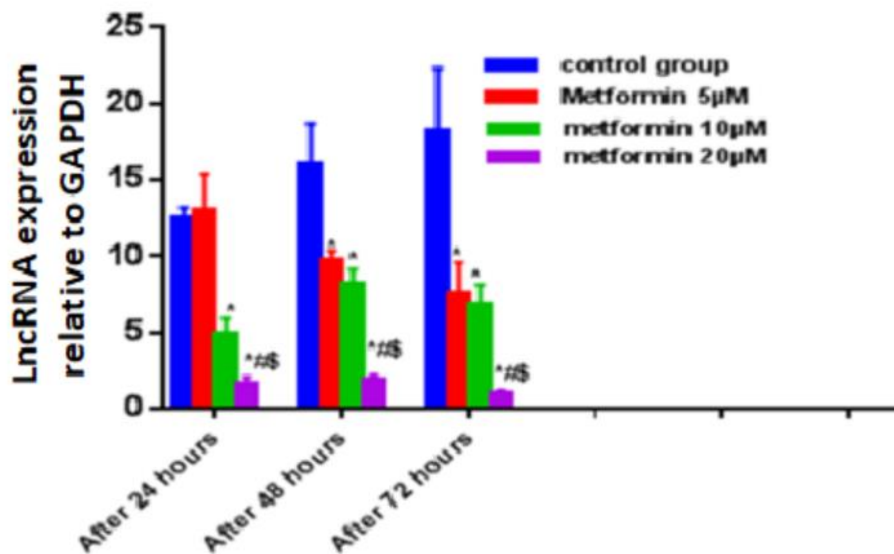

Fig. 5: lncRNA-AF085935 relative gene expression (*Mean ± SD)* among various studied groups.
p value <0.05 was significant. (*) Denotes a significant difference against control group of the same duration. (#) Denotes a significant difference versus Metformin 5µMgroup of the same duration. ($) Denotes a significant difference versus Metformin 10µM group of the same duration.

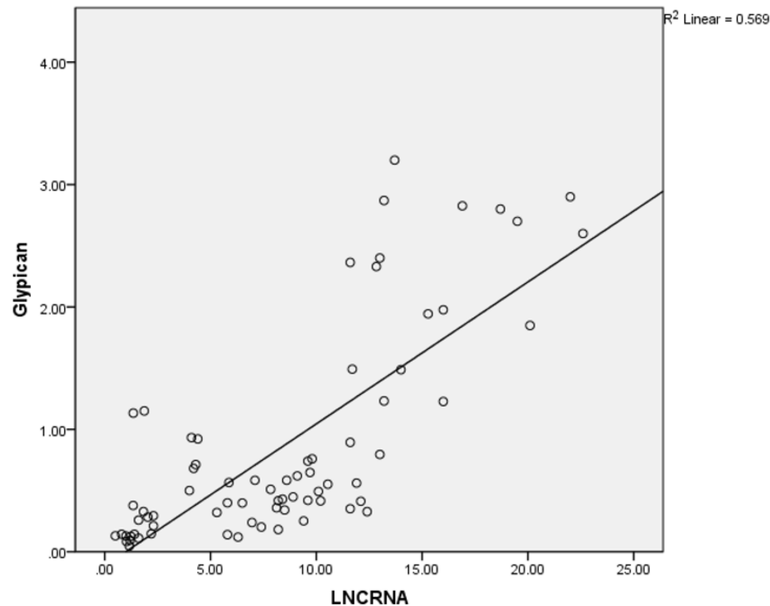

Fig. 6: Correlation between Glypican-3 gene expression and LncRNAAF085935 expression among various studied groups.

Concerning MAPK protein level, we have measured the ratio between the phosphorylated p44/42 and the total p44/42 and found a significant decrease in the protein level in treated groups (versus the control group) (p value <0.0001) except for 5µM-24h group (p value >0.05). It was noted that the 10µM-24h and 20µM-24h groups showed less significance (p values 0.02, and 0.001) respectively (after comparing the corresponding concentrations of higher duration with their control values), supporting the duration-dependent effect of metformin on MAPK (Fig. 7).

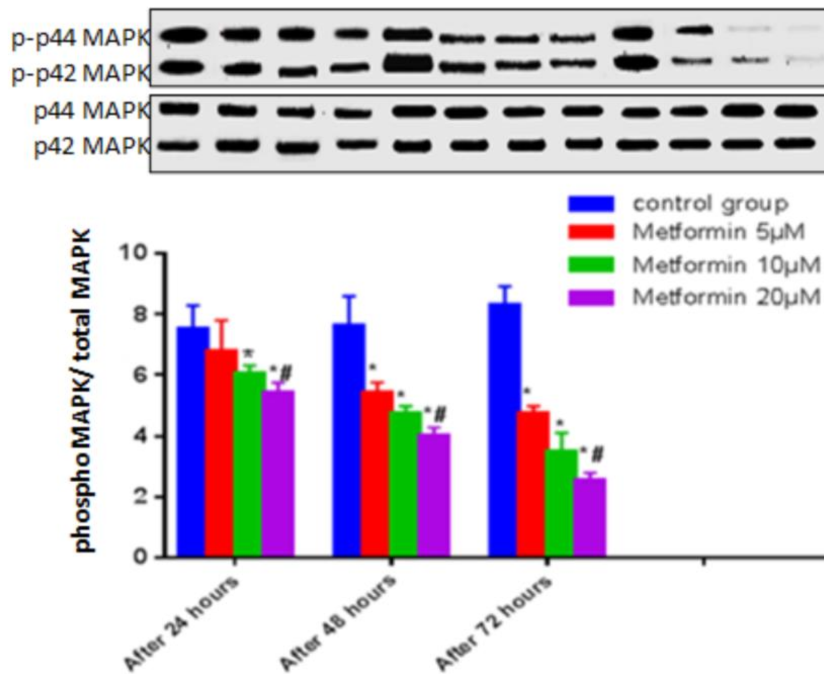

Fig. 7: Western blotting analysis of p-p44/42 MAPK and the total p44/42 MAPK protein levels (*Mean ± SD)* among various studied groups.
p value <0.05 is significant. (*) means a significant difference versus control group of the same duration. (#) means a significant difference versus Metformin 5µMgroup of the same duration.

VEGF is the principal driver of angiogenesis. In our study, we found a significant decrease in VEGF relative gene expression in all metformin-treated groups (p value <0.001) except for the metformin 5µM-24h group (p value = 0.9). The latter showed significantly higher VEGF gene expression relative to the 20µM-24h group (Fig. 8**).**

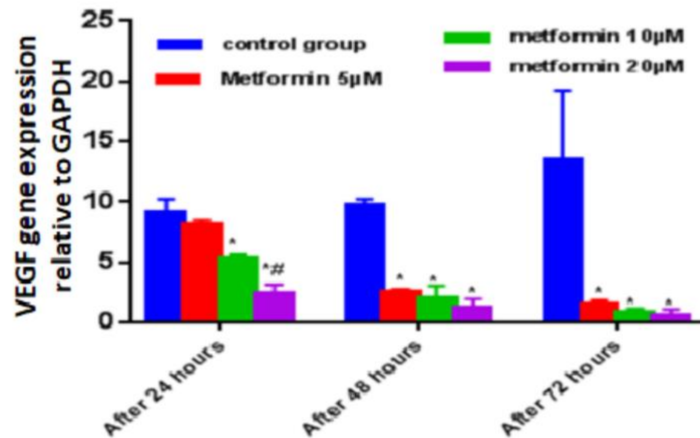

Fig. 8: VEGF gene expression (*Mean ± SD)* among various studied groups.
p value <0.05 was significant. (*) means a significant difference versus control group of the same duration. (#) means a significant difference versus Metformin 5µMgroup of the same duration.

Regarding apoptosis, we assessed p53 gene expression and found a dose- and time-dependent significant increase in P53 gene expression in the 20µM treated cells compared to the corresponding control cells of the same duration. This defined group also showed a significant increase if compared to the correspondingly lower concentrations only after 48 and 72h **(**Fig. 9). It is worthy of note that the 5µM group showed no significant difference in any duration.

As regards nuclear factor κB (NFκB) gene expression, our study showed a significant decrease in NFκB gene expression in all groups treated with metformin compared to the control group (p value <0.001) after 48 and 72 h only **(**Fig. 10).

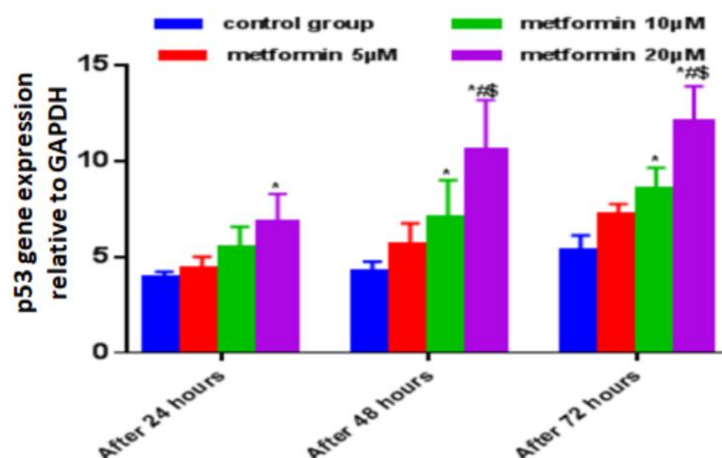

Fig. 9: P53 gene expression (*Mean ± SD)* among various studied groups
p value <0.05 was significant. (*) Denotes a significant difference versus control group of the same duration. (#) Denotes a significant difference versus Metformin 5µMgroup of the same duration. ($) Denotes a significant difference versus Metformin 10µM group of the same duration.

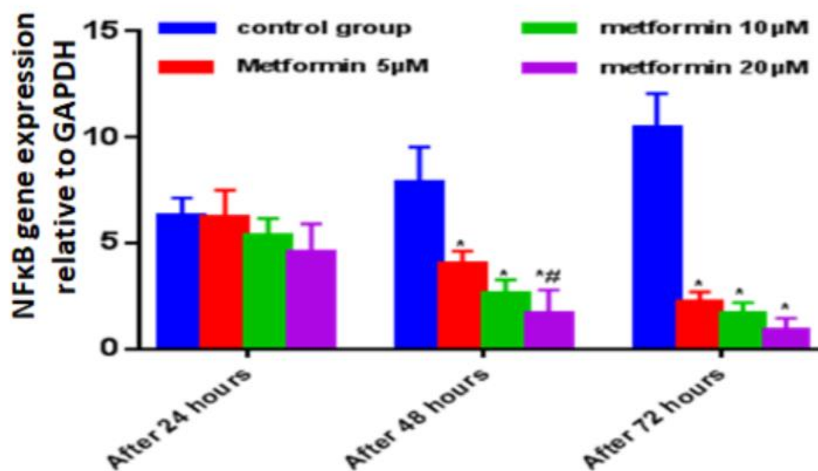

Fig. 10: NFκB gene expression (*Mean ± SD)* among various studied groups.
p value <0.05 was significant. (*) Denotes a significant difference versus control group of the same duration. (#) Denotes a significant difference versus Metformin 5µMgroup of the same duration.

DISCUSSION

Despite treatment breakthroughs, hepatocellular carcinoma (HCC) remains among the most lethal malignancies globally. The antidiabetic multitarget drug metformin is gaining more attention for its anticancer effects against HCC development. Following intestinal absorption, metformin in the most commonly prescribed doses of 1 to 2g/day leads to plasma concentrations of 10 to 40µM (Kajbaf *et al.,* 2016). However, most studies dealing with metformin in cancer were conducted at 10- to 100 times higher concentrations than maximally practicable therapeutic ones (He and Wondisford, 2015).

Based on our curiosity about metformin, the current study was designed to explore whether therapeutic doses of metformin (5, 10, and 20µM) at different durations (24, 48, and 72h) could affect LncRNA-AF085935/Glypican-3 and MAPK pathway in HCC in vitro.

Regarding cell proliferation, our results exposed a significant decrease in all metformin-treated groups except the metformin 5µM-24h group, supporting the dose- and time-dependent effects of metformin on cell proliferation. We share consistent results of these low-dose effects with a few other studies (Sabry *et al.,* 2019), (Sun *et al.,* 2016), (Zhang *et al.,* 2017). Similar studies, but with higher concentrations of metformin (in millimoles) applied to HCC, also showed harmonious results (Cai *et al.,* 2013), (Miyoshi *et al.,* 2014), (Ferretti *et al.,* 2019), (Tawfik *et al.,* 2022). Other studies using metformin on other types of cancer cell types confirm the same concept, including surgically resected glioblastoma tissue samples (Guarnaccia *et al.,* 2022) and human gastric cancer cells (Lu *et al.,* 2019).

Concerning the LncRNA-AF085935/GPC3 axis, both parameters showed significantly independent decreased levels in the treated groups, especially after 48 and 72h and they revealed a strong positive correlation with one another. In agreement with us, Sabry *et al.,* 2019 determined a significant decrease in both GPC3 protein expression level and LncRNA-AF085935 expression in HepG2 cancer cells after supplementation with EGCG and metformin, illustrating that metformin is more effective than EGCG on this lncRNA. Those can support Lu et al., 2015, who assumed that LncRNA-AF085935 could be a possible biomarker for HCC. The effect of metformin on decreasing GPC3 expression may aggravate the inhibitory effect of metformin on insulin-like growth factors (IGFs) and their binding proteins as GPC3 induces

oncogenicity by preventing IGF-1R degradation (Cheng *et al.,* 2017).

Concerning MAPK protein level, our study revealed a significant decrease in p-p44/42 MAPK protein level in treated groups except for 5µM-24h group and less significance in the 10µM-24h and 20µM-24h groups, supporting the duration-dependent effect of metformin on p-p44/42 MAPK. GPC3 may enhance p44/42 MAPK activation, promoting the epithelial-mesenchymal transition (EMT) of HCC cells (Wu *et al.,* 2015). A mechanism that clarifies the GPC3/MAPK link is that GPC3 interacts with FAT1, an integral protein on the cell surface of HCC cells, and acts concomitantly to regulate the expression of EMT-related genes Snail, Vimentin, and E-Cadherin and promote HCC (Meng *et al.,* 2021). GPC3, in addition, has the ability to bind to IGF-II along with IGF-1 receptors (IGF-1R), triggering phosphorylation of IGF-1R as well as the subsequent downstream signaling molecule p44/42 MAPK (Cheng *et al.,* 2008).

Blankly, GPC3 had another behavior in breast cancer, as it was found that GPC3 reverses the EMT, inhibits metastasis, and enhances dormancy of cancer cells by activating p38 MAPK and that p-p44/42/phospho-p38 ratio is lower in GPC3 re-expressing cells (Guereño *et al.,* 2020). The hierarchical relationships among different MAPK pathways and GPC3 remain to be determined.

The anti-carcinogenic effect of metformin on MAPK follows the same line as NFκB gene expression, which revealed a significant decrease in all groups treated after 48 and 72h. That results support a previous report (Hsieh *et al.,* 2014) where metformin inhibited the invasion of human HCC cells and boosted the chemosensitivity to sorafenib via downregulating the ERK/JNK in an NF-κB-based pathway. In addition, millimolar concentrations of metformin attenuate p44/42 phosphorylation/ anti-apopototic signaling as previously reported in gastric cancer (Lu *et al.,* 2019), MCF-7 breast cancer cell line (Malki and Youssef, 2011), papillary thyroid carcinoma cells (García-Sáenz *et al.,* 2022), ovarian cancer cells (Dang *et al.,* 2017), and A549 lung cancer cells (Dong *et al.,* 2020), (Zhang *et al.,* 2018). Contrastingly to our results, He *et al.,* 2021 found that metformin can affect MAPK signaling via inhibiting stimulation of p38 MAPK and JNK MAPK but not via p44/42 MAPK and rescue insulin secretion in type 2 diabetes. They used both in vivo diabetic rat models and in vitro rat pancreatic β-cells. This conflict may be caused by the use of different species or by the fact that the effects of metformin on MAPK signaling may differ in cancers.

Metformin decreases the vascularization of HCC cells in a dose- and duration-dependent way in our study, as we found a significant decrease in VEGF gene expression in all treated groups except 5µM-24h group, inhibiting its angiogenic effect. This result supports the previous results of Sabry *et al.,* 2019 on metformin-treated HepG2 cells and Dang *et al.,* 2017 after applying higher concentrations of metformin to the human ovarian HO-8910 cancer cell line.

Regarding apoptosis, we found a significant increase in P53 gene expression, especially with 20µM after 48 and 72h. Metformin allows augmentation of p53 and its putative stabilization through its action on adenosine monophosphate-activated protein kinase (AMPK) (Ferretti *et al.,* 2019) as metformin is a well-known inhibitor of complex I of the respiratory chain, resulting in consequent activation of AMPK (Skuli *et al.,* 2022). This effect can be augmented if combined with antifolates (Miyoshi *et al.,* 2014) and can be mediated through miR-23a, a tumor suppressor induced by metformin (Sun *et al.,* 2016).

Conclusion

Taken together, these studies and our present findings suggest that low doses of metformin (10 and 20µM) may have the potential to suppress HCC by decreasing viability and division of cancer cells, downregulating MAPK, Glypican-3, LncRNA-AF085935, VEGF, and NF-κB gene expression, and upregulating P53 gene expression, while the effect of metformin

5µM, when applied for a short duration (24h) is still a subject of debate. Findings that need further investigation by functional assays in multiple cell lines or through in vivo studies in the future

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Competing Interests: The authors have no relevant interests to disclose.

Authors Contributions: Investigation: Radwa Taha, Mona Said, Naglaa Fathy. Project administration and resources: Dina Sabry Software: Mona Said. All authors contributed to the study conceptualization, design, validation, writing, editing, and approval of the final manuscript.

Ethics Approval: The study was approved by the Faculty of Medicine, Cairo University, Egypt.

Data Availability: All data engendered during this work are encompassed in this manuscript.

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ARABIC SUMMARY

دور محتمل للميتفورمين في سرطان الخلايا الكبدية: دراسة في المعمل لمسار LncRNA-AF085935 / Glypican-3 / MAPK

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* المؤلف المراسل

يعد سرطان الخلايا الكبدية من أكثر أنواع السرطان انتشارا في العالم حيث يعد سرطان الخلايا الكبدية خامس السرطانات الاكثر تشخيصا والسبب الرئيسى الثالث للوفاة بالسرطان في جميع انحاء العالم. يعتبر سرطان الخلايا الكبدية واحد من أكثر أنواع السرطان انتشارا في مصر و عادة ما يكون سببه تليف الكبد بسبب العدوى الفيروسية خصوصا عدوى فيروس التهاب الكبد الوبائي(سي) .وعلي الرغم من التقدم الكبير في تشخيص وعلاج سرطان الخلايا الكبدية فأن توقعات سير مرض سرطان الخلايا الكبدية لا يزال ضعيفا ويرجع ذلك الي حد كبير الي المراحل المتقدمة في الوقت الذي يتم فيه تشخيصه وارتفاع معدلات تكراروانتشار الورم الخبيث.كما تظل الاثار الجانبية للعلاج الكيميائي ومقاومة الادوية من الشواغل الرئيسية لخيارات العلاج .لذلك تركز استراتيجيات العلاج الكميائي الجديدة علي خيارات جديدة مع عدد اقل من الاثار الجانبية لمنع نمو الاورام الموجودة ومنع غزو الحلايا السرطانية وتكوين الاوعية الدموية.

يستخدم عقار الميتفورمين كخيار أول لعلاج النوع الثاني لمرض البوال السكري مع تقارير عن فائدته للوقاية والسيطرة على عدة أنواع من السرطانات.

تهدف هذه الدراسة إلى تقييم تأثير الجرعات المنخفضة من عقار المتفورمين علي الانتشار وموت الخلايا المبرمج وتولد الاوعية الدموية في سرطان الخلايا الكبدية.

أجريت الدراسة في المختبر علي خط خلية سرطان خلايا الكبد حيث تمت معالجة خلايا الكبد السرطانية بتركيزات مختلفة من عقار الميتفورمين (5و10و20 ميكرو مولار) علي فترات زمنية مختلفة (24و48و72 ساعة) مقابل مجموعات التحكم الغير معالجة بالعقار.

تم تقسيم الدراسة الي اربعة مجموعات:

المجموعة الاولى (الضابطة): وتضم خلايا الكبد السرطانية الغير معالجة المستزرعة لمدة (24و48و72 ساعة).

المجموعة الثانية : وتضم خلايا الكبد السرطانية المعالجة بتركيز (5 ميكرو مولار) من عقار الميتفورمين ومستزرعة لمدة (24و48و72ساعة).

المجموعة الثالثة: وتضم خلايا الكبد السرطانية المعالجة بتركيز (10 ميكرو مولار) من عقار الميتفورمين ومستزرعة لمدة (24و48و72ساعة)..

المجموعة الرابعة: وتضم خلايا الكبد السرطانية المعالجة بتركيز(20 ميكرو مولار) من عقار الميتفورمين ومستزرعة لمدة (24و48و72ساعة).

تم عمل الاتى لمجموعات الدراسة:

1. قياس معدل تكاثر خلايا الكبد السرطانية باختبار الام ام تي.
2. قياس معدل التعبير الجينى الكمى لكل من الجينات التالية (جلابكن3وحمض الريبونيوكليك الطويل الغير مشفر أ-أ ف 539580 وعامل نمو بطانة الاوعية الدموية وبي 53 والعامل النووي كابا بي) بتحليل البلمرة المتسلسل العددى.
3. تقدير العلاقة بين التعبير الجيني الكمي لجين الجلابكن3 والتعبير الجيني الكمي لحمض الريبونيوكليك الطويل الغير مشفر أ-أ ف 539580.
4. تم تقييم النسبة بين مستوى البروتين 42 / p44 الفسفوري ومستوى البروتين الكلي 42 / p44

نتائج الدراسة:

1. نقص ملحوظ في معدل تكاثر الخلايا في كل المجموعات المعالجة بعقار الميتفورمين مقارنة بالمجموعة الضابطة في المدد الزمنية الثلاثة المختلفة (24و48و72 ساعة) باستثناء ادني تركيز (5 ميكرومولار) الذي لم يظهر دلالة في اقصر مدة وهي 24 ساعة.لذلك يمكن للميتفورمين ان يقلل من حيوية وقدرة الخلايا علي التكاثرمعتمدة علي الجرعة والوقت.
2. هناك انخفاض ملحوظ في التعبير الجيني لجين الجلابكن3 في جميع المجموعات المعالجة بعقار الميتفورمين مقارنة بالمجموعة الضابطة في 48و72 ساعة .في حين لا يوجد اي فرق كبير يذكر في 24 ساعة.

3. هناك انخفاض ملحوظ في التعبير الجيني لحمض الريبونيوكليك الطويل الغير مشفر أ-أ ف 539580 في كل المجموعات المعالجة بعقار الميتفورمين مقارنة بالمجموعة الضابطة في المدد الزمنية الثلاثة المختلفة (24و48و72 ساعة) باستثناء ادني تركيز (5 ميكرومولار) الذي لم يظهر دلالة في اقصر مدة وهي 24 ساعة.
4. هناك انخفاض ملحوظ في التعبير الجيني لبروتين 42 / p44 الفسفوري وكذلك عامل نمو بطانة الاوعية الدموية في كل المجموعات المعالجة بعقار الميتفورمين مقارنة بالمجموعة الضابطة في المدد الزمنية الثلاثة المختلفة (24و48و72 ساعة) باستثناء ادني تركيز (5 ميكرومولار) الذي لم يظهر دلالة في اقصر مدة 24 ساعة.
5. هناك زيادة ملحوظة في التعبير الجيني لجين بي 53 في المجموعات المعالجة بتركيز 10و20ميكرومولار واضحة مع زيادة المدة ولكن لم تظهر المجموعة المعالجة بتركيز 5ميكرومولار مثل هذه الزيادة.
6. هناك انخفاض ملحوظ في التعبير الجيني للعامل النووي كابا بي في المجموعات المعالجة بعقار الميتفورمين مقارنة بالمجموعة الضابطة في (48و72ساعة) في حين لا يوجد اي فرق يمكن ذكره في 24 ساعة.
7. هناك ارتباط جيد جدا بين مستوي التعبير الجيني لجين جلابكن3 و التعبير الجيني لحمض الريبونيوكليك الطويل الغير مشفر أ-أ ف 539580($p < 0.001$)

الخلاصة

ترجح الدراسة الحالية أن عقار الميتفورمين له تأثير علاجي علي سرطان الخلايا من خلال حمض الريبونيوكليك الطويل الغير مشفر أ-أ ف 539580 / جين جلابكن3 / بروتين 42 / p44